

### **REMARKS**

The specification has been amended to more clearly define the product identified by the trademark PLURONIC®F68. Specifically, the specification has been amended to recite that the specified block copolymer has an average molecular weight of 8400 Da. Support for this amendment is based on what was conventional and well known to one of ordinary skill in the art (see, e.g., Murhammer et al., 1988, Bio/Technology 6:1411-1418, "Murhammer", at pg. 1411, col. 2, first paragraph; and Wu, 1995, J Biotechnology 42:81-94, "Wu", at page 87, paragraph spanning col. 1 and col. 2) and is restricted to the characteristics of the product known at the time the application was filed. See MPEP §§ 608.01(v) and 2163 (citing *Hybritech Inc. v. Monoclonal Antibodies, Inc.* 802 F.2d 1367).

Claims 1, 3-16, and 18-47 were pending in the present application.

Claims 8, 9, 23, 24, and 31-46 have been canceled without prejudice. Claims 3-5 and 18-20 have been amended without prejudice. Applicants reserve the right to prosecute the deleted subject matter in one or more continuing applications.

Claims 3-5 and 18-20 have been amended to clearly define the product identified by a trademark. Specifically, claims 3-5 and 18-20 have been amended to recite a polyoxyethylene-polyoxypropylene block copolymer having an average molecular weight of 8400 Da. Support for this amendment can be found in the specification, for example, in the paragraph beginning on page 4, line 30, as amended herein.

New claims 48 and 49 have been added directed to a specific embodiment wherein the concentration of cell-lysing component is less than 0.00025%. Applicants believe that support for this amendment can be found in the specification, for example, at page 9, lines 25-33, page 13, lines 25-26 and page 29, lines 5-8 (PS-80 is 1% of a 0.25% final concentration of buffer A).

Applicants respectfully submit that no new matter has been added by these amendments.

After entry of the foregoing amendments, claims 1, 3-7, 10-16, 18-22, 25-30, and 47-49 will be pending.

**Rejection under 35 U.S.C. § 112, Second Paragraph**

Claims 3-5, 10-15, 17-20 and 23-30 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, the Examiner contends that claims 3-5, 10-15, 17-20 and 23-30 are indefinite because the claims contain the trademark/trade name Pluronic® F-68.

Without admitting to the propriety of the rejection and in an effort to advance prosecution, Applicants have amended claims 3-5 and 18-20 to recite the product identified by trademark/trade name Pluronic® F-68, i.e., a polyoxyethylene-polyoxypropylene block copolymer having an average molecular weight of 8400 Da.

Applicants respectfully submit that the rejection has been obviated. Accordingly, Applicants respectfully request withdrawal of the rejection.

**Rejection Under 35 U.S.C. § 103**

Claims 1, 3-16, 18-30 and 47 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Wu in view of Brough et al. (U.S. Pat. No. 6,113,913, "Brough") and Murhammer. Specifically, the Examiner contends that it would have been obvious to perform the scale-up of mammalian cell culture for the production of adenovirus vectors using Pluronic®F-68 because (1) one of skill in the art would have been motivated to propagate adenoviral vectors on a large scale because of their usefulness as gene delivery vehicles (as disclosed in Brough) and to use Pluronic®F-68 in the large scale method (because Wu discloses that Pluronic®F-68 is a protective medium additive that can protect animal cells during gas sparging); and (2) one of skill in the art would have had a reasonable expectation of success that Pluronic®F-68 would have had a protective effect on animal cell infected with adenovirus during gas sparging because Murhammer teaches the propagation of insect cells on a large scale using Pluronic®F-68 and that Pluronic®F-68 had been known to protect mammalian cells in a sparged environment. Applicants respectfully traverse.

The present invention, as exemplified in claims 1 and 16, is drawn to a method of large scale virus production in mammalian cells cultured under gas sparging, which has two essential features: (a) use of cell growth medium containing an effective amount of a shear-

protective compound that is a block copolymer surfactant; and (b) use of virus seed stocks essentially free of any cell-lysing component.

Wu discloses that mechanical damage of suspended animal cells during mechanical agitation and sparging aeration is associated with gas bubbles. See Wu, page 82, col. 1, first full paragraph. Wu also discloses that the presence of chemical additives such as Pluronic®F-68 can increase the tolerance of animal cells to stressful mechanical forces. See *id.* at page 82, col. 1, second full paragraph. Wu does not discuss the effect of chemical additives such as Pluronic®F-68 on the production of viruses.

Brough discloses a recombinant adenovirus lacking E1 gene expression and having a mutated major late promoter. See Brough, col. 3, lines 48-50. Brough further discloses that modified adenoviruses are a superior and safe vehicle for gene transfer (see *id.* at col. 1, lines 9-27) and can be propagated in Per.C6® cells (see *id.* at col. 9, lines 43-45). Brough does not discuss the use of medium additives such as Pluronic®F-68 for virus production.

Murhammer discloses the scale-up of insect cell cultures and further demonstrates the protective effects of Pluronic®F-68 concentrations in sparged cultures of insect cells and the production of a recombinant protein in insect cells infected with baculovirus. See Murhammer, pg. 1411, abstract. Murhammer demonstrates that growth of insect cells (uninfected with virus) in sparged cultures in the presence of Pluronic®F-68 was comparable to that in a 50 ml spinner flask. See *id.*, pg. 1413, col. 1, third full paragraph. In contrast, after infection with virus, very little cell growth occurred and viral titers peaked around 25 hours. See *id.*, pg. 1413, paragraph spanning col. 1 and 2. Notably, Murhammer did not see any improvement in viral titer with increasing concentrations of Pluronic®F-68 and saw reduced protein production compared to nonsparged cultures. Murhammer suggests that higher concentrations of Pluronic®F-68 are needed to fully protect virally infected cells, but saw no improvement at 0.5% Pluronic®F-68. See *id.*, pg. 1414, col. 2, first full paragraph.

First, Applicants respectfully submit that there is no suggestion or motivation to combine Wu, Brough and Murhammer. The Examiner's contention that one of skill in the art would have been motivated to propagate adenoviral vectors on a large scale (based on Brough) and to use Pluronic®F-68 in the large scale method (based on Wu) fails to take into account all the teachings of the prior art. Applicants note that the Examiner's citations in Murhammer relate

only to the growth of uninfected cells. As Applicants have pointed out, little is known about the shear-sensitivity of infected mammalian cell cultures. See the specification, at page 5, lines 9-15.

The observation in Murhammer that after infection with virus, very little cell growth occurs and there is a significant drop in cell viability casts doubt on the usefulness of Pluronic®F-68 in virus production. Increasing the concentration of Pluronic®F-68 failed to fully-protect virally-infected cells from lysis. However, these results can be difficult to interpret since viral titer was not measured in the absence of Pluronic®F-68.

Notably, when considering the prior art as a whole<sup>1</sup>, Pluronic®F-68 has been shown to have a significant negative impact on virus production. See Palomores et al., 2000, *Enzyme Microb Technol* 26:324-331 ("Palomores"; reference C01). Palomores found that compared to controls lacking Pluronic®F-68, the use of Pluronic®F-68 in insect cultures infected with baculovirus increased protein production ten-fold but decreased virus production twenty-fold. See id., abstract, Table 1, and Figure 3. In the presence of Pluronic®F-68, viral titer dramatically slowed after 24 hours<sup>2</sup>. See id., Figure 3. In the absence of Pluronic®F-68, viral titers continued to increase up to 72 hours and were twenty fold higher. See Palomores, Figure 3. These data are more relevant than that of Murhammer in that viral production was directly measured and provide a strong teaching away from using Pluronic®F-68 in virus production.

Thus, when considering the teachings of the prior art, as exemplified by Murhammer and Palomores, one of skill in the art would not find a suggestion or motivation to use a block copolymer surfactant such as Pluronic®F-68 in a large scale method for the production of virus.

Second, Applicants respectfully submit that Wu in view of Brough and Murhammer do not provide a reasonable expectation of success.

With respect to the use of a block copolymer surfactant such as Pluronic®F-68 in large-scale production virus, in view of the teachings of Palomores, the cited references cannot

---

<sup>1</sup> "The test for obviousness is what the combined teachings of the references would have suggested to one of ordinary skill in the art, and all teachings in the prior art must be considered to the extent that they are in analogous arts. Where the teachings of two or more prior art references conflict, the examiner must weigh the power of each reference to suggest solutions to one of ordinary skill in the art, considering the degree to which one reference might accurately discredit another." MPEP § 2143.01 citing *In re Young*, 927 F.2d 588, 18 USPQ2d 1089 (Fed. Cir. 1991).

provide a reasonable expectation of success because the prior art showed that the use of Pluronic®F-68 significantly reduced the production of virus.

With respect to the use of virus seed stocks essentially free of any cell-lysing component, there is no basis for applying the teachings of baculovirus production in insect cells to the large-scale production of virus in mammalian cells. Baculovirus production and harvesting in insect cells occurs in cell culture media and buffers without cell-lysing components. In contrast, production of viruses in mammalian cells occurs in cell culture media and lysing buffers containing cell-lysing components. See Xie et al. Biotechnol Bioeng 83:45-52 ("Xie"; reference C02), abstract. Moreover, it was unexpected that the presence of a cell-lysing component (PS-80) at a concentration of 0.00025% would have such an impact on virus production. See id.

Thus, one of skill in the art would not have a reasonable expectation of success for a large-scale method of virus production combining (a) use of cell growth medium containing an effective amount of a shear-protective compound that is a block copolymer surfactant; and (b) use of virus seed stocks essentially free of any cell-lysing component.

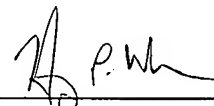
For the above reasons, Applicants respectfully request withdrawal of the rejection.

### CONCLUSION

Applicants respectfully submit that all claims are in condition for allowance and earnestly solicit a favorable action on the merits.

Respectfully submitted,

Date: August 23, 2007

By   
Henry P. Wu  
Reg. No. 44,412  
Attorney for Applicant  
MERCK & CO., INC.  
P.O. Box 2000  
Rahway, New Jersey 07065-0907  
(732) 594-5312

---

<sup>2</sup> This result is similar to that seen in Murhammer. See Murhammer, Figure 4.